MEASUREMENT OF CARBON DIOXIDE EVOLUTION FROM AERATED SLUDGE

By Nandor Porges, Lenore Jasewicz, and Sam R. Hoover

Eastern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Dept. of Agriculture, Philadelphia, Pa.

The rate and extent of the oxidizing ability or activity of an aerated sludge may be measured in the simple manner described herein. The direct measurement of oxygen uptake of a sludge requires manometric methods with apparatus and technique not generally available for routine practice. On the other hand, the described measurement of the amount of CO_2 evolved from a sludge-substrate mixture is easy, direct, and relatively rapid. It offers a tool for ascertaining the potency of the sludge and should find application.

The possibility of utilizing CO_2 production as a measurement of sludge activity devolved from a prior study on the assimilation of dairy waste (3). That investigation was made by means of the Warburg apparatus. Solutions of skim milk (1,000 p.p.m.), lactose (500 p.p.m.), and casein (350 p.p.m.) each had its own rate and extent of oxidation when acted upon by the sludge organisms. The activity was essentially complete in 6 hr. Analyses showed that 60 to 68 per cent of the substrate was converted to cell material, whereas the remainder was oxidized.

Determinations of the respiratory quotient (R.Q.) for each of these systems demonstrated that the volume of CO_2 evolved was practically equal to the oxygen consumed; that is, R.Q. = $CO_2/O_2 = 1$. Thus, it appeared obvious that under a highly aerobic environment, the CO_2 evolved may serve as a measure of sludge activity.

Apparatus

A simple device for the measurement of CO_2 evolution from sludge is pictured in Figure 1. The principles of the apparatus are not novel. A similar apparatus finds use in studying the microorganisms in soil (1). In the latter case, days or weeks are required, whereas with aerated sludge the test is completed in a short while. Briefly, CO_2-free air is passed through the sludge-substrate mixture and the spent CO_2-laden air is bubbled through barium hydroxide solution. The air is forced through under pressure or vacuum. In the studies here reported compressed air was available at 20 lb. gage pressure.

A list of the materials used in this apparatus follows:

- 1 Drying tower or calcium chloride cylinder.
- 1 Flowmeter.
- 1 Quart Mason jar with removable ring of a two-piece Mason cap.
- 1 Rubber stopper No. 13 with 3 holes.
- 1 Tube, gas dispersion; with fritted disc, Pyrex No. 39525/20M.
- 1 Calcium chloride drying tube, 100 mm.
- 1 Funnel, separatory, cylindrical, 125 ml.
- 2 Culture tubes, 25 by 300 mm.
- 1 Culture tube, 25 by 200 mm.
- 2 Tubes, gas dispersion, with fritted cylinders, Pyrex No. 39533/12C.
- 1 Tube, glass with Rose bulb at one end.

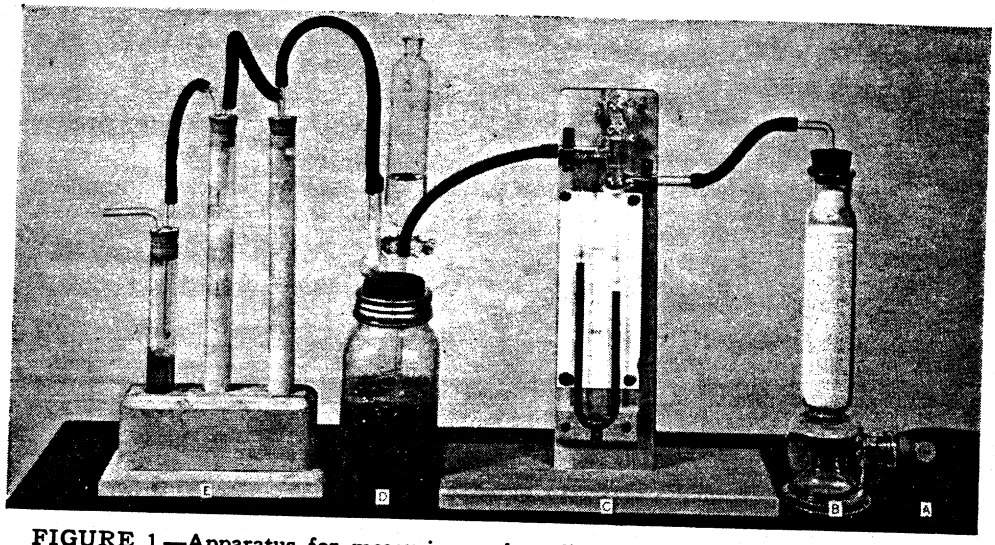

FIGURE 1.—Apparatus for measuring carbon dioxide evolution from aerated sludge substrate mixtures. A, air regulating valve; B, soda-lime tower; C, flowmeter; D, quart jar aerator; E, tubes containing $Ba(OH)_2$.

Rubber stoppers and tubing as required.
Silicone, D–C antifoam A.
Soda-lime, 4 to 8 mesh.

In addition, the following reagents are required:

$Ba(OH)_2$, 0.1N, containing 8.57 g. anhydrous or 15.78 g. hydrous per liter.
Oxalic acid, 0.1N, containing 6.3 g. of the hydrate per liter.

Details are shown in Figure 1. The flow of compressed air is regulated by the needle valve (A). The air passes up through the tower (B) containing soda-lime for CO_2 removal. The CO_2-free air is measured by means of the flowmeter (C) and regulated by valve (A) to give a rate of flow of one volume per volume of sludge mixture per minute (500 ml. air per 500 ml. mixture per minute). The air is dispersed in the 500 ml. of sludge-substrate mixture contained in the quart jar (D). The gas dispersion tube with fritted disc serves as the sparger. The spent air, carrying evolved CO_2, leaves the jar through the upright tube containing glass wool and enters the three tubes (E) containing 25 ml. 0.1N $Ba(OH)_2$, water, and phenolphthalein. (The glass wool prevents passage of liquid droplets.) Thorough removal of the CO_2 by the $Ba(OH)_2$ is secured by using the two gas dispersion tubes with fritted cylinders in the first two test tubes, as shown. The third contains the tube with the Rose bulb; that is, the end of the tube is blown to form a small bulb and is perforated with three or four holes.

Figure 1 shows a single outfit. In most of the experiments at least five were used at one time. In that case, the air scrubbing tower was replaced by a 40-in. length of 2-in. pipe attached to the compressed air line. This held about 2 lb. of soda lime. The CO_2-free air was distributed through flowmeters by means of a manifold equipped with needle valves. The air also may be prepared by bubbling through caustic solution.

Procedure

Place 140 ml. of settled sludge in the jar. Insert the three-hole stopper with the assembly in the mouth of the jar and fasten in place with the Mason

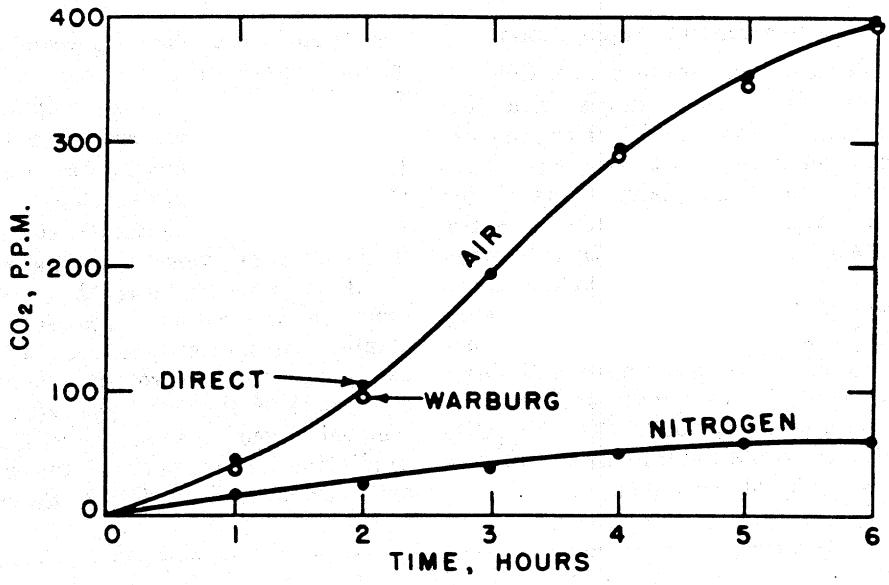

FIGURE 2.—Carbon dioxide production from skim milk in air and in nitrogen.

cap ring. Connect the sparger tube with the flowmeter. Pass CO_2-free air through the apparatus for 15 min. to sweep out residual CO_2. Stop the air flow. Add 360 ml. sewage or substrate through the separatory funnel to give a final volume of 500 ml. Close the glass cock. Connect the upright spent air tube with the three tubes containing the $Ba(OH)_2$ solution. Pass air through the apparatus and regulate the rate of flow. Add silicone antifoam to aerating mixture and to the $Ba(OH)_2$ tubes. Aerate for 1 hr. and replace the $Ba(OH)_2$-containing tubes with another set of tubes containing 25 ml. of $Ba(OH)_2$ solution. Permit air to flow during the interchange in order to avoid back passage of the solution. Transfer the contents of the three tubes to a 500-ml. flask and titrate with the oxalic acid to the colorless phenolphthalein end point. Replace the tubes containing $Ba(OH)_2$ at hourly or other desired intervals. Calculate the results and plot the data. The ratio of sludge to sewage may be varied to simulate operating conditions.

Calculations

The spent air from the aerator is relieved of the CO_2, thus

$$CO_2 + Ba(OH)_2 \rightarrow BaCO_3 + H_2O.$$

Hence, 1 ml. 0.1N $Ba(OH)_2$ = 1 ml. 0.1N CO_2 = 2.2 mg. CO_2. The $BaCO_3$ precipitates as an insoluble salt. The excess $Ba(OH)_2$ is titrated by the oxalic acid, which does not react with the $BaCO_3$. The difference in value between the $Ba(OH)_2$ present at the beginning in the 25 ml. of standard solution and that found by the oxalic acid gives the amount of $Ba(OH)_2$ combined with CO_2

An example follows:

25 ml. $Ba(OH)_2$, 0.1181N, at start	= 29.52 ml. 0.1N
15.4 ml. oxalic acid, 0.1343N, back titration	= 20.68 ml. 0.1N
$Ba(OH)_2$ carbonated, by difference	= 8.84 ml. 0.1N
CO_2 produced per aerator of 500 ml.	= 19.45 mg.
CO_2 produced, mg. per liter or	38.9 p.p.m.

Experimental Applications

Comparisons between the CO_2 obtained from the aerators with that calculated from the direct oxygen uptake measured manometrically have been made using dairy waste sludge and various substrates (5). Results showed excellent correlation between these two methods. Some of the values were plotted in Figure 2. Replacing air by nitrogen gas inhibited the activity of the sludge, retarded the oxidation of the skim milk, and resulted in reduced formation of CO_2. The CO_2 obtained with a water blank was practically negligible (4 p.p.m.)

was used in the Warburg vessel. The results presented in Figure 2 have taken the dilutions into consideration.

A peculiarity occurred when a 0.35 per cent casein solution was used instead of the skim milk solution. The CO_2 calculated from the O_2 consumed in the Warburg vessel was consistently about twice as great as that evolved from the jar aerators. Apparently, the alkaline substances produced by the oxidation of the proteins combined with some of the CO_2, preventing its removal from the aerating sludge. Acidifying the contents of the jar to pH 3 or lower with H_2SO_4 after the

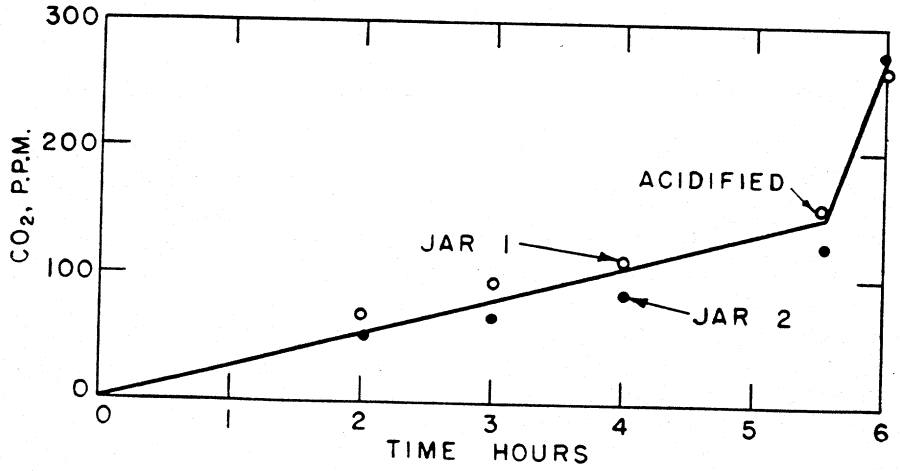

FIGURE 3.—Carbon dioxide evolved from casein showing importance of acidification after aeration period.

Some tests were made using 500 ml. of aerated diluted dairy waste sludge in the quart jar (D) and 3 ml. in the Warburg vessel. The total chemical oxygen consumed was 416 p.p.m., of which 42 p.p.m. was present in the supernatant solution after centrifuging. By difference, the chemical oxygen consumed by the solids was 374 p.p.m., which was calculated as 298 p.p.m. of bacterial cells using the factor of 1.25 g. O_2 required to oxidize per gram of dry cells (3). Fifty ml. of 1 per cent skim milk solution were added without additional dilution to the aerator (D) through the separatory funnel; 0.3 ml.

aeration period and then continuing aeration for 30 min. gave the results shown in Figure 3. The CO_2 output was practically doubled. When using a highly proteinaceous material or one that may produce an alkaline condition, acidification will be necessary at the end of the aeration period to obtain a true value for the CO_2 production. Use of buffers may be desirable in some cases.

Some experiments were made with municipal sludge and sewage obtained locally, through the courtesy of R. M. Bolenius of the Abington Township (Pa.) sewage treatment plant. The

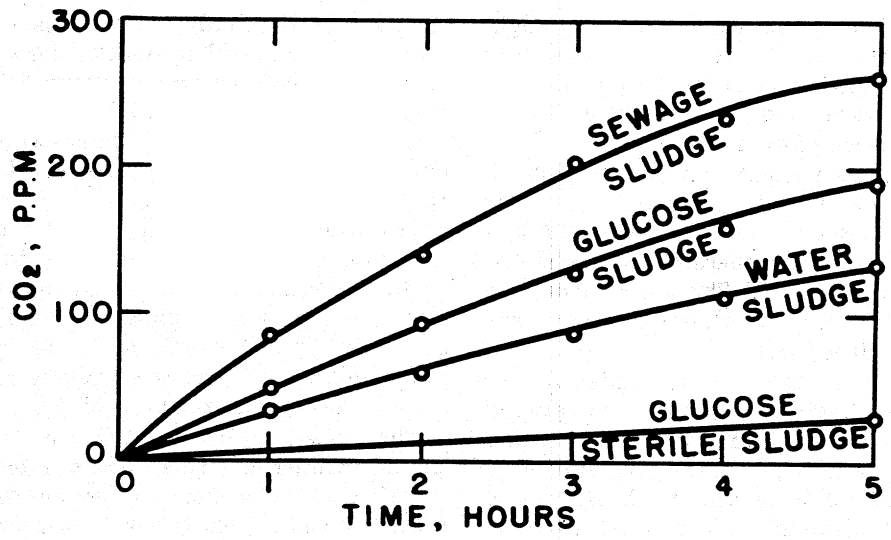

FIGURE 4.—Evolution of carbon dioxide from 40 parts municipal sludge plus 100 parts substrate.

CO_2 evolved from 40 parts of fresh settled sludge and 100 parts sewage was compared to that in which the sewage was replaced by glucose and by water. To 140 ml. of fresh sludge placed in the jar, 360 ml. of sewage, glucose solution, or water were added. The sludge-sewage mixture was very active in the liberation of CO_2. The sewage had a chemical oxygen consumed value of 288 p.p.m. The 200-p.p.m. glucose-sludge mixture was less active. A later test showed that CO_2 production was increased slightly by the addition of $(NH_4)_2SO_4$. When water replaced the sewage, activity was still marked, but reduced about one-half; apparently the sludge has large amounts of available nutrients. Heat-sterilized sludge, to which the glucose

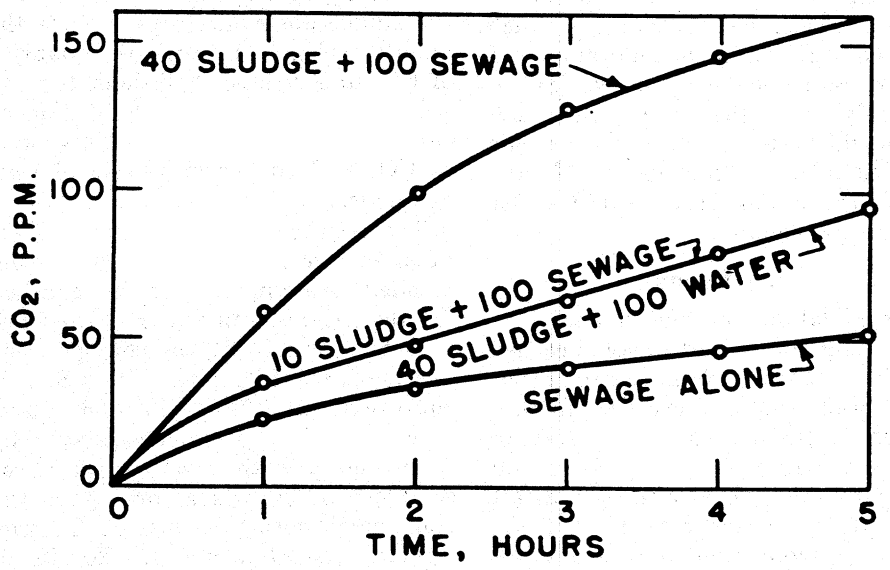

FIGURE 5.—Carbon dioxide formed by 500 ml. of sewage-sludge mixtures.

solution was added, showed only slight activity. In this experiment, the water blank had a value of 53 p.p.m. The data, corrected for the water blank, have been plotted in Figure 4.

An experiment in which heat-sterilized sewage was added to fresh sludge and to heated sludge gave values of 63 p.p.m. CO_2 liberated from the latter in 5 hr. and 282 p.p.m. from the former. A viable sludge was necessary to produce CO_2.

Using freshly obtained materials, the ratio of sludge to sewage was altered in order to note the effect of decreasing the quantity of sludge. To 100 parts of sewage were added 40, 10, and 0 parts of sludge. To another 40 parts of sludge were added 100 parts of water. The total volume added to each aerator was 500 ml. The differences in activity were readily obvious from the plotted CO_2 data (Figure 5). In this test the soda lime in the absorption chamber had been changed and a low value of 10 p.p.m. CO_2 was obtained with the water blank.

The manometric Warburg experiments were conducted at 30° C. with the close control of temperature required for such measurements. The titrimetric experiments described were carried out at room temperature, which was about 27° C. in the laboratory. If the laboratory temperature varies appreciably (more than 2° C.) the quart jar should be placed in a water or air thermostat so that the activity of various sludges would be measured under comparable conditions.

Discussion

The oxidation occurring in a mixture of aerating sludge and waste is caused by the activity of microorganisms. These organisms produce CO_2 in proportion to the amount of O_2 used in obtaining energy for synthesizing cell material. The CO_2 evolved is a direct index of the sludge activity. If the sludge is sluggish and the waste is attacked slowly, CO_2 production is retarded. If the sludge activity is destroyed, there is little or no CO_2 formation. Such destruction may occur with toxic wastes. An active sludge with nutrients balanced, as in dairy waste or household sewage, is associated with rapid and high CO_2 evolution. The greater the amount of sludge used with the sewage, the faster the latter is utilized and the more rapid is CO_2 formation. Conversely, smaller amounts of sludge produced CO_2 more slowly. Likewise, an insufficiency of oxygen results in decreased amounts of CO_2.

The measurement of CO_2 in the simple manner described offers a tool of value in many phases of waste disposal. Within a short time (as short as 1 hr. in some cases) considerable information may be obtained about the sludge and about the sewage. The oxidizing ability of the sludge may be measured and an indication obtained as to the amount required for mixing with the influent. The amount of sewage to be added to sludge and the necessity of supplemental nutrients may be determined. The amount of sludge solids produced from a waste in the aeration process may be calculated also. In addition, information may be obtained concerning the oxygen requirements of the sludge-waste mixtures. Since the quantity of oxygen equals the quantity of CO_2 under these conditions, the volume of O_2 required per milligram of cells per hour is equal to the volume of CO_2 evolved per milligram of cells per hour.

The results of the few experiments upon sewage indicate that the recommended procedure could be adapted for the determination of B.O.D. Thus in 5 hr. (Figure 5) the sewage alone has produced 50 p.p.m. CO_2, which is equivalent to $0.727 \times 50 = 36$ p.p.m. O_2 consumed. Oxygen consumption by the rapid growth reaction would be complete in 3 days according to the manometric measurements of Ludwig, Oswald, and Gotaas (4). Results of like nature were obtained by Gellman

and Heukelekian (2) with a Sierp apparatus. The slower endogenous respiration of the organisms would require many days to reach a negligibly slow rate, just as is required for the conventional B.O.D. determination. Further investigation of this application would seem to be a promising line of research.

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